EMBO reports

Calcineurin promotes APC/C activation at meiotic exit by acting on both XErp1 and Cdc20

Andreas Heim, Thomas Tischer, Thomas U. Mayer

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 20 February 2018

Thank you for the submission of your research manuscript to EMBO reports. We have now received reports from the three referees that were asked to evaluate your study, which can be found at the end of this email.

As you will see, all referees think the manuscript is of interest, but requires further revisions to allow publication in EMBO reports. All three referees have a number of concerns and/or suggestions to improve the manuscript, which we ask you to address in a revised manuscript. As the reports are below, and I think all the points need to be addressed, I will not detail them here. Please also provide the quantifications of the experiments as indicated by referees #1 and #3.

Given the constructive referee comments, we would like to invite you to revise your manuscript with the understanding that all referee concerns must be addressed in the revised manuscript and in a detailed point-by-point response. Acceptance of your manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss the revisions further.

Supplementary/additional data: The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf labeled Appendix. The Appendix includes a table of content on the first page, all figures and their legends. Please follow the nomenclature Appendix Figure Sx throughout the text and also label the figures according to this nomenclature.

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Important: All materials and methods should be included in the main manuscript file.

See also our guide for figure preparation:
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Regarding data quantification and statistics, can you please specify, where applicable, the number "n" for how many independent experiments (biological replicates) were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. Please provide statistical testing where applicable. See:
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Please also follow our guidelines for the use of living organisms, and the respective reporting guidelines: http://embor.embopress.org/authorguide#livingorganisms

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When submitting your revised manuscript, we will require:

- a complete author checklist, which you can download from our author guidelines (http://embor.embopress.org/authorguide#revision). Please insert page numbers in the checklist to indicate where the requested information can be found.
- a letter detailing your responses to the referee comments in Word format (.doc)
- a Microsoft Word file (.doc) of the revised manuscript text
- editable TIFF or EPS-formatted single figure files in high resolution (for main figures and EV figures)

Please also note that we now mandate that all corresponding authors list an ORCID digital identifier that is linked to their EMBO reports account!

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

REFEREE REPORTS

Referee #1:

The manuscript by Heim et al. aims to shed light on the controversial role of the phosphatase Calcineurin in release from CSF arrest in oocytes, using Xenopus laevis as a model system. Using CSF extracts, the authors show that Calcineurin promotes in addition to the kinase CaMKII efficient response to calcium stimulus to exit meiosis. Calcineurin seems to be required at lower Calcium levels, which is probably important under more physiological conditions, even though CaMKII is the essential player for release from CSF arrest. The contributions of Calcineurin are more subtle, and on the one hand required for phosphorylation of Xerp1 on sites required for its degradation, and on the other hand for the dephosphorylation of Cdc20, which is phosphorylated on inhibitory sites in metaphase II. Overall, the study is well executed, and very careful analysis of subtle differences observed upon inhibition of Calcineurin allowed the authors to analyse these novel roles of Calcineurin in meiotic exit.

I have the following minor remarks only:

In Figure 1A and Figure EV1 the differences in Cyclin B2 levels between control and CaN inhibitor treated oocytes after release are very low. Could the authors provide quantifications of several experiments to strengthen the data?

The authors could show a stronger exposure of the Cyclin B2 western blot in Figure 1C to better appreciate the difference at 300 and 400 μM calcium. (the bands are hardly visible on the figures I have at my disposition)

The difference in the degradation of endogenous and exogenously expressed Xerp1 in Figure 1E would be strengthened by quantifications.

Given the fact that Calcineurin seems to be the main phosphatase dephosphorylating the inhibitory phosphorylation site on Cdc20 I would expect a stronger effect upon inhibition of Calcineurin on cexit from CSF arrest. Do the authors know which fraction of endogenous Cdc20 is phosphorylated on this site? Alternatively, do the authors think that there is another phosphatase required in addition to Calcineurin?

In Figure 3B, why is there no more Cdc20 present in later time points upon CsA addition, like in the control?

Calcineurin was shown to destabilize Bora, leading to the inactivation of Plk1. At exit from CSF arrest, Plx1 is required for phopho-degron dependent degradation of Xerp1. Can the authors speculate on how Calcineurin may promote exit from meiosis II by dephosphorylating Cdc20 while not interfering with Plx1 activity?

Referee #2:

In this paper the role of the phosphatase calcineurin (CaN) in regulating meiotic exit is explored. It is already established that a calcium wave leads to the activation of APC/C-Cdc20 to drive exit and previous work has implicated CaMKII and CaN as important regulators but the exact function of CaN has been elusive. Here the authors identify two important targets of CaN during meiotic exit - XErp1 (an APC/C-Cdc20 inhibitor) and Cdc20 (the APC/C co-activator). The authors nicely demonstrate that CaN dephosphorylates sites in XErp1 that allows for the binding of PP2A-B56 and that this help to destabilize XErp1 removing part of the brake on APC/C-Cdc20. Furthermore they show that CaN also target Cdc20 inhibitory sites to further facilitate APC/C-Cdc20 activity and that the threonine preference of CaN is important for controlling temporal activation.

Although some of the effects are subtle due to multiple pathways coming together to activate APC/C-Cdc20 I think the experiment in figure 1C is telling in that the effects can be controlled by Calcium levels and therefore one is convinced that these uncovered mechanisms are likely to be very important in the animal.

Overall the experiments are well executed and the manuscript well structured and a coherent story presented and suitable for EMBO reports.

A few suggestions for improvement:

The one thing that would have been nice is to have more insight into how CaN recognizes XErp1 and Cdc20. Could it be that CaN binds an APC/C subunit (a quick look indicates that there are possible CaN docking motifs in APC1 and APC3). Maybe it would be worth seeing if CaN is present in APC/C IPs or vice versa or if immunodepletion of APC/C affects CaN targeting of Cdc20. A long the same line is CaN recognizing the entire XErp1-Cdc20-APC/C complex or do the authors have data to suggest differently (XErp1 or Cdc20 dephosphorylation kinetics in absence of different components)

It should be possible to engineer the B56 binding site in XErp1 so it is no longer regulated by phosphorylation and therefore not by CaN. In such a mutant does this bypass the regulation of

XErp1 stability by CaN - this would be an alternative experiment since they cannot detect XErp1-B56 interaction. Motif in XErp1: LSTLREQSSQS could be engineered to LPTIREEEEQS but off course this could have other effects.

Minor things:

1) Page 3: do the authors mean human/mouse Thr55, Thr59...
2) A schematic of XErp1 primary structure and motifs they mutate/delete would be really helpful as part of Figure 1.

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•The manuscript, somehow, contradicts findings of Nishiyama et al. where CaN inhibition blocks cyclin B degradation completely for at least an hour. Here, the difference in cyclin B degradation between controls and CaN-inhibited extracts is marginal. Could the authors comment on this?

•In many cases, the differences between controls and treated extracts are not very obvious or difficult to read in the blots. It might be helpful to include graphic representations of the blots (i.e. for 1B cyclin, 1E cyclin and xErp1, 2A slow and fast migrating forms, 3B slow and fast migrating forms of Cdc20 and Cdc27).

•The authors suggest that CaN constitutes an auxiliary pathway that supports CaMKII and that conditions that hamper CaMKII action should increase the contribution of CaN to meiotic exit. The authors present a number of indirect experiments to verify this hypothesis. I was wondering whether direct inhibition of CaMKII leads to an increased participation of CaN in meiotic exit (CAMKII inhibitors +/- CsA)

•In Figs. 2D and 3D the authors show, in an in vitro system, the efficiency of CaN to induce specific dephosphorylation events in xErp1 and Cdc20 respectively. Occasionally, in vitro and in vivo systems behave differently. Is it possible to show the dephosphorylation efficiency of CaN in extracts in the absence of calcium (constitutively active CaN)?

•In Fig. 3B, CaN inhibition seems to block the dephosphorylation of Cdc27. Could the authors speculate on this?

•The evaluation of the role of CaN in dephosphorylating xErp1 and Cdc20 is shown by the use of the CaN inhibitor CsA. Another inhibitor (FK506) is only shown in regards to cyclin B degradation and its effect on cyclin B degradation does not seem to be strong (Fig. EV1). Are the CsA effects on xErp1 and Cdc20 replicated by the use of FK506 or other means of CaN inhibition?

1st Revision - authors' response 20 May 2018

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I have the following minor remarks only:

In Figure 1A and Figure EV1 the differences in Cyclin B2 levels between control and CaN inhibitor treated oocytes after release are very low. Could the authors provide quantifications of several experiments to strengthen the data?

We agree and included for Figure 1B a quantification of Cyclin B2 levels in DMSO- and CsA-treated extracts from six independent experiments (*new* Fig. EV1A). To strengthen the point that FK506 – like cyclosporine A (CsA) – impairs APC/C activation at calcium-induced exit from meiosis, we replaced old EV1A with a new figure showing the effect of FK506 on Cyclin B2 destruction at various calcium concentrations (*new* Fig. EV1B).

The authors could show a stronger exposure of the Cyclin B2 western blot in Figure 1C to better appreciate the difference at 300 and 400 µM calcium. (the bands are hardly visible on the figures I have at my disposition)

We now included a short and long exposure of the Cyclin B2 western blot (*new* Fig. 1C).

The difference in the degradation of endogenous and exogenously expressed Xerp1 in Figure 1E would be strengthened by quantifications.

We appreciate this comment and included now a quantification of the levels of endogenous and exogenous XErp1 (*new* Fig. EV1E).

Given the fact that Calcineurin seems to be the main phosphatase dephosphorylating the inhibitory phosphorylation site on Cdc20 I would expect a stronger effect upon inhibition of Calcineurin on exit from CSF arrest. Do the authors know which fraction of endogenous Cdc20 is phosphorylated on this site? Alternatively, do the authors think that there is another phosphatase required in addition to Calcineurin?

Unfortunately, the phospho-specific antibodies are not sensitive enough to detect endogenous Cdc20 and we therefore cannot make any conclusion about the fraction of phosphorylated Cdc20. We agree with the reviewer that – in addition to CaN – further phosphatases are likely to act on Cdc20. One piece of evidence relates to the timing of CaN activity and Cdc20 dephosphorylation: as observed by us and others (Nishiyama et al, *Nature*, 2007; Mochida and Hunt, *Nature*, 2007) CaN is only active for a very short time window (app. 3 – 6 min) in oocyte extract after calcium-induced meiotic exit and Cdc20 dephosphorylation continues beyond this narrow activity window of CaN (e.g. see Fig. 3B, 16-24 min). Furthermore, it has been shown that PP2-B55 (Hein et al, *Nature Cell Biology*, 2017) and PP1 (Kim et al, *Genes Dev*. 2017) dephosphorylate Cdc20 in somatic human and *C. elegans* cells, respectively. Thus, as suggested by Hunt & Mochida it is likely that CaN acts as an initiating phosphatase that breaks the cell cycle arrest at fertilization resulting in the activation of further phosphatases that continue to act on Cdc20.

In Figure 3B, why is there no more Cdc20 present in later time points upon CsA addition, like in the control?

In Fig. 3B, we used Phos-tag™ SDS-PAGE to maximally resolve the phosphorylated forms of Cdc20. The differentially phosphorylated forms of Cdc20 present in CsA-treated extracts might have given the wrong impression that Cdc20 levels decline under these conditions. In the revised version of our manuscript, we therefore included an immunoblot of a regular (non-Phos-tag™) SDS-PAGE to demonstrate that Cdc20 levels remained constant during the experiment (*new* Fig. 3B).

Calcineurin was shown to destabilize Bora, leading to the inactivation of Plk1. At exit from CSF arrest, Plx1 is required for phopho-degron dependent degradation of Xerp1. Can the authors

speculate on how Calcineurin may promote exit from meiosis II by dephosphorylating Cdc20 while not interfering with Plx1 activity?

This is an interesting point. As shown by Feine et al (*Cell Cycle* 2014), in Xenopus oocyte extract Plx1 inactivation takes place about 40 min after the calcium-induced exit from meiosis. In contrast, Cdc20 dephosphorylation and XErp1 destruction take place within 20 min and 10min, respectively, after calcium addition (Figs. 3B and 1E). We therefore speculate that CaN-mediated Bora degradation does not immediately result in Plx1 inactivation, which requires its dephosphorylation at an activating Aurora-A site. Such a delay in Plx1 inactivation would provide a time window where CaN can contribute to APC/C activation by removing inhibitory phosphorylations on Cdc20 and by promoting XErp1 destruction via the CaMKII/Plx1/$^{SCF\beta\text{-}TRCP}$ axis before Plx1 is inactivated.

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In this paper the role of the phosphatase calcineurin (CaN) in regulating meiotic exit is explored. It is already established that a calcium wave leads to the activation of APC/C-Cdc20 to drive exit and previous work has implicated CaMKII and CaN as important regulators but the exact function of CaN has been elusive. Here the authors identify two important targets of CaN during meiotic exit - XErp1 (an APC/C-Cdc20 inhibitor) and Cdc20 (the APC/C co-activator). The authors nicely demonstrate that CaN dephosphorylates sites in XErp1 that allows for the binding of PP2A-B56 and that this help to destabilize XErp1 removing part of the brake on APC/C-Cdc20. Furthermore they show that CaN also target Cdc20 inhibitory sites to further facilitate APC/C-Cdc20 activity and that the threonine preference of CaN is important for controlling temporal activation.

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A few suggestions for improvement:

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We agree with the reviewer that more insights into how CaN recognizes XErp1 and Cdc20 would have been nice. Our in vitro assays suggest that CaN can directly dephosphorylate XErp1 and Cdc20. However, we do not exclude the possibility that additional factors might be involved. Since phosphorylation of Cdc20 prevents it from binding to the APC/C (Hein and Nilsson, *Nat Com*, 2016; Labit et al., *EMBO J*, 2012), we would assume that the dephosphorylation of Cdc20 by CaN occurs in an APC/C-independent manner. Of course, it could be possible that phosphorylated Cdc20 still binds weakly to the APC/C and that APC/C-associated CaN dephosphorylates Cdc20. For XErp1 we also have currently no data supporting the idea that CaN associated with the APC/C dephosphorylates XErp1. As mentioned before, additional insights into CaN-mediated dephosphorylation of Cdc20 and XErp1 would be nice, but we are afraid that such studies would go beyond the scope of this manuscript. We amended the main text to emphasize the possibility that additional factors might be involved in the dephosphorylation of Cdc20 and XErp1.

It should be possible to engineer the B56 binding site in XErp1 so it is no longer regulated by phosphorylation and therefore not by CaN. In such a mutant does this bypass the regulation of XErp1 stability by CaN - this would be an alternative experiment since they cannot detect XErp1-

B56 interaction. Motif in XErp1: LSTLREQSSQS could be engineered to LPTIREEEEQS but off course this could have other effects.

This is a great suggestion. We mutated the B56 binding motif of XErp1 to LPTIREEEEQS and this results – as postulated by the reviewer – in a hyperbinding mutant (*new* Fig. EV2C). Importantly, CaN inhibition had no effect on the phosphorylation state of the hyperbinding mutant (*new* Fig. EV2D, please note that we used stable XErp1 (ΔDSG, ΔDSA) in order to be able to analyse the phosphorylation state uncoupled from XErp1 destruction).

Minor things:

1) Page 3: do the authors mean human/mouse Thr55, Thr59...

Yes, we refer to both human and mouse Cdc20 and changed the text accordingly.

2) A schematic of XErp1 primary structure and motifs they mutate/delete would be really helpful as part of Figure 1.

This is a great suggestion and we added a scheme of the different XErp1 constructs used in this study in the *new* Fig. 2A.

Referee #3:

The manuscript of Heim et al. attemps to elucidate the specific roles of the phosphatase calcineurin in the activation of the frog oocyte. The specific calcineurin-dependent dephosphorylation events that affect xErp1 and Cdc20 are identified. The manuscript presents novel and interesting findings. However, I think that further data is needed in order to determine accurately the importance of calcineurin for oocyte activation. I would like the following comments to be addressed by the authors:

•The manuscript, somehow, contradicts findings of Nishiyama et al. where CaN inhibition blocks cyclin B degradation completely for at least an hour. Here, the difference in cyclin B degradation between controls and CaN-inhibited extracts is marginal. Could the authors comment on this?

The effect of CaN inhibition on cyclin B destruction varies from a strong (Nishiyama et al, *Nature*, 2007) to a modest stabilization of cyclin B (Mochida and Hunt, *Nature*, 2007). Thus, we agree that the effect we observe is not as severe as the one reported by Nishiyama et al, but is rather comparable to the one observed by Mochida and Hunt. While we have no explanation for this variability, we also noticed that the effect of CaN inhibition on cyclin B destruction – while being reproducible – varies in its strength from extract to extract. To take this into account, we added new figures showing the quantifications of cyclin B2 levels from independent replicates (*new* Figs. EV1A and EV1E)

•In many cases, the differences between controls and treated extracts are not very obvious or difficult to read in the blots. It might be helpful to include graphic representations of the blots (i.e. for 1B cyclin, 1E cyclin and xErp1, 2A slow and fast migrating forms, 3B slow and fast migrating forms of Cdc20 and Cdc27).

We agree and therefore added quantifications for cyclin B2 and XErp1 destructions (*new* Figs. EV1A and EV1E). To emphasize the slow and fast migrating forms of Cdc20 and Cdc27, we pseudo-coloured the different bands according to their signal intensity using a linear image analysis tool of the Fujifilm Multi-Gauge V3.0 software (*new* Figs. EV2A and EV4A).

•The authors suggest that CaN constitutes an auxiliary pathway that supports CaMKII and that conditions that hamper CaMKII action should increase the contribution of CaN to meiotic exit. The authors present a number of indirect experiments to verify this hypothesis. I was wondering whether direct inhibition of CaMKII leads to an increased participation of CaN in meiotic exit (CAMKII inhibitors +/- CsA)

This is a good suggestion. We used an inhibitory peptide ($CaMK^{281-309}$) at different concentrations to interfere with CaMKII activation. In line with our previous data and as suggested by the reviewer, direct inhibition of CaMKII results in an increased impact of CaN on meiotic exit (*new* Fig. EV1C).

•In Figs. 2D and 3D the authors show, in an in vitro system, the efficiency of CaN to induce specific dephosphorylation events in xErp1 and Cdc20 respectively. Occasionally, in vitro and in vivo systems behave differently. Is it possible to show the dephosphorylation efficiency of CaN in extracts in the absence of calcium (constitutively active CaN)?

We performed the suggested experiment and supplemented oocyte extract with mRNA encoding constitutively active calcineurin (ca-CaN). However, in line with previous publications (Mochida and Hunt, *Nature*, 2007), expression of ca-CaN was not sufficient to induce meiotic exit or to trigger the dephosphorylation of XErp1 or Cdc20. We speculate that in the presence of high Cdk1 activity, ca-CaN alone is not capable of promoting the efficient dephosphorylation of Cdc20 and XErp1 required to break the strong cell cycle arrest.

•In Fig. 3B, CaN inhibition seems to block the dephosphorylation of Cdc27. Could the authors speculate on this?

The reviewer is right in that CaN inhibition interferes with dephosphorylation of Cdc27. This effect has already been observed by Hunt and Mochida (*Nature*, 2007, Supp. Fig. S1). Yet, the dephosphorylation of Cdc27 occurs at a timepoint when CaN is again inactivated: CaN is active for about 3 – 6 min after calcium stimulation (see Nishiyama et al, *Nature*, 2007; Mochida and Hunt, *Nature*, 2007), while Cdc27 dephosphorylation starts app. 12 min after calcium stimulation (Fig. 3B). This indicates that CaN indirectly affects Cdc27 dephosphorylation. An obvious link for such an indirect mechanism is phosphatase inhibitor-1 (I-1), which in its phosphorylated form inhibits protein phosphatase 1 (PP1). As shown previously (Mulkey et al., *Nature*, 1994), CaN dephosphorylates I-1 in postsynaptic cells and this results in increased PP1 activity. In Xenopus oocyte extracts, dephosphorylation of I-1 upon calcium stimulation occurs exactly during the time window of CaN activity (Hunt and Mochida, *Nature*, 2007, Supp. Fig. S2J) suggesting that phosphorylated I-1 is also a substrate of CaN in meiotic cells. Activated PP1 reverses the phosphorylation of many Cdk1 substrates by both activating the phosphatase PP2A-B55 – a key antagonist of Cdk1 – (Heim et al, *EMBO R*, 2015) and by directly dephosphorylating Cdk1 substrates. Thus, these data suggest that CaN transiently activated at fertilization antagonizes Cdk1 phosphorylations by multiple pathways including APC/C activation (via Cdc20 and XErp1 dephosphorylation) and also by activating downstream phosphatases (e.g. PP1) which ultimately convert the cell cycle machinery (e.g. the APC/C) in its dephosphorylated interphasic form.

•The evaluation of the role of CaN in dephosphorylating xErp1 and Cdc20 is shown by the use of the CaN inhibitor CsA. Another inhibitor (FK506) is only shown in regards to cyclin B degradation and its effect on cyclin B degradation does not seem to be strong (Fig. EV1). Are the CsA effects on xErp1 and Cdc20 replicated by the use of FK506 or other means of CaN inhibition?

This is a good suggestion. We analysed the effect of FK506 addition on the dephosphorylation of XErp1 and Cdc20. In line with our previous results, the appearance of the fast migrating, i.e. dephosphorylated, form of Cdc20 upon calcium-addition was delayed in FK506-treated extract compared to the control (*new* Fig. EV4B). In the case of XErp1, addition of FK506 mimicked the effect of CsA in that the dephosphorylation of XErp1 was accelerated upon calcium-induced exit from meiosis (*new* Fig. EV2B).

2nd Editorial Decision 23 July 2018

Thank you for the submission of your revised manuscript to our editorial offices. We have now received the reports from the three referees that were asked to re-evaluate your study (you will find enclosed below). As you will see, the referees now fully support the publication of your manuscript in EMBO reports.

Before we can proceed with formal acceptance, I have the following editorial requests that we ask you to address in a final revised version of the manuscript:

- Could statistical testing be performed for the diagrams shown in Figs. EV1A and EV1E? Could you then also add a paragraph describing the statistical testing used?

- For Fig. EV2A and EV4A, please show also the unprocessed blot together with the pseudocolored one.

- Please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text, and some queries (comments), we ask you to address. Please provide your final manuscript file with track changes, in order that we can see the modifications done.

I look forward to seeing the final revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

REFEREE REPORTS

Referee #1:

The authors have addressed all my concerns in a satisfying manner. In my opinion the revised manuscript should be accepted for publication in Embo Reports.

Referee #2:

The authors have addressed my concerns and in my view also that of the other reviewers. Furthermore the schematic added to Figure 2A is very useful. I recommend publication.

Referee #3:

All my comments have been addressed by the authors in this revised manuscript and therefore I support the publication of the manuscript.

2nd Revision - authors' response 30 July 2018

The authors performed all minor editorial changes.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Thomas U. Mayer
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2018-46433V1

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	NA
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	All CSF extracts prepared from oocytes of Xenopus laevis were assesed prior to experiments by testing their ability to maintain a stable metaphase state in the absence of external calcium and to undergo meiotic exit in its presence. The results of these tests were used as quality criteria to include or exclude CSF extracts from the following experiments. All experiments contained appropriate controls that were used to judge if CSF extract integrity was maintained during the experiments.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	All treatments/conditions of one experiment were performed with aliquots of the same CSF extract that was prepared from a single, randomly chosen frog. All experiments were repeated with CSF extracts prepared from different individual frogs.
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	All results shown are primary data, with the exception of the immunoblot quantifications in Fig. EV1A and EV1E. The immunoblot quantifications were performed with the ImageJ software in an identical procedure for all conditions. The investigators were unblinded when assessing the results, as is standard in the field for the kind of experiments performed here.
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	NA
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	NA
Is there an estimate of variation within each group of data?	Standard deviations were calculated and are shown as error bars for the immunoblot quantifications in Fig. EV1A and EV1E.
Is the variance similar between the groups that are being statistically compared?	NA

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	For all commercially available antibodies used in this study, we included the provider and catalog number (see materials and method (M&M) section). For previously published antibodies (XErp1 and Cdc27), we refer to the publications (see M&M section). The pS335 XErp1 and pT68 Cdc20 antibodies are characterized in Fig. EV3A-C and Fig EV4C, respectively. The purification procedure of the Flag antibody is described in the M&M section, its specificity for immunoprecipitations is confirmed by using a different commercial Flag antibody for the immunoblots.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Female Xenopus laevis frogs were bred and maintained under laboratory conditions at the animal research facility, University of Konstanz and all procedures performed were approved by the Regional Commission, Freiburg, Germany.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	Female Xenopus laevis frogs were used for the production of metaphase II arrested eggs. These procedures were approved by the Regional Commission, Freiburg, Germany. Subsequent experiments were performed with extracts prepared from these eggs.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	Source data for all immunoblots and the individual data points of the immunoblot quantifications are provided.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NO